Acute traumatic coagulopathy: Whole blood thrombelastography measures the tip of the iceberg

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BACKGROUND: Thrombelastography (TEG) is suggested as an optimal instrument for the identification of acute traumatic coagulopathy–induced alterations in coagulation status. Patient whole blood (WB) used in TEG analysis is generally collected from a large blood vessel containing representative systemic blood, often close to 40% hematocrit (Hct). Trauma patients often exhibit bleeding from the microvasculature. This study examines early coagulation function changes at the simulated microvascular level based on altered Hct and pH in vitro through TEG analyses of normal donor blood.

METHODS: Anticoagulated normophysiologic fresh human blood was centrifuged. Individual component effects on coagulation were investigated through variable recombination groups: platelet-rich plasma (PRP), platelet-poor plasma (PPP), and red blood cells (RBCs), which were compared with WB. Acute traumatic coagulopathy–induced acidic microvascular environment was simulated and investigated using tissue factor–activated TEG analysis of variable Hct (40%, 30%, 20%, and 0%) samples and variable [H^+]. Incremental replacement of RBC with either PPP or normal saline (NS) simulated resuscitation in vitro was also conducted under similar conditions.

RESULTS: Only acidified PRP reflected loss of clot strength. Acidified PRP and PPP were delayed equally in clot time. In all groups, inclusion of RBCs normalized clot time. RBC replacement with PPP significantly delayed clot time when samples were acid-challenged, signifying greater acid effect in low Hct microvascular beds. NS simulated resuscitation incurred even greater clotting delays.

CONCLUSION: Acidemia-induced coagulopathy at the level of the capillary Hct (1) is more severe than at higher Hct levels (larger blood vessels), (2) shows that simulated resuscitation with NS causes greater increases in clot time and decreases in clot strength beyond that which occurs with plasma replacement, and (3) may not accurately be portrayed through common TEG practice of testing systemic WB of greater than 30% Hct. (*J Trauma Acute Care Surg*. 2015;78: 955–961.

KEY WORDS: Erythrocyte; red blood cells; acidemia; thrombelastography; tissue factor.

Trauma is the leading cause of mortality for patients 44 years and younger.[1] In 1995, causes of death were divided into 42% central nervous system injury, 39% exsanguinations, and 7% organ failure.[2] The military literature suggests that 90% of trauma-induced, potentially survivable mortality is caused by exsanguination,[3,4] thereby overwhelmingly weighting the importance of hemorrhage control following combat trauma. A major contributing factor to hemorrhage mortality is the development of a coagulopathy in the setting of significant tissue injury and shock. This bleeding phenomenon is classified as acute traumatic coagulopathy (ATC). ATC occurs in approximately 25% of trauma patients[5] and may manifest as abnormal coagulation within 30 minutes following traumatic wounding,[6] a time span and condition not associated with significant fibrinogen consumption and before iatrogenic hemodilution caused by resuscitative efforts.[7] The international normalized ratio (INR), which strongly correlates with a lack of adequate tissue blood flow in surgical and trauma patients,[8] is the most commonly used indicator of ATC. However, information provided by the INR, while more sensitive to coagulation factor activity and thrombin generation, is correlative at best and does not reflect platelet function or red blood cell (RBC) influence. Thus, to better treat ATC, we must understand its mechanisms and be able to more accurately diagnose its presence in trauma patients.

Thrombelastography (TEG) is commonly used in the clinical setting in diagnosis of ATC. TEG, per manufacturer's instructions using tissue factor (TF) or kaolin coagulation activation, measures elapsed time to clot initiation, rate of fibrin cross-linkage, strength of clot formed, and rate of fibrinolysis of a whole blood (WB) sample, a perceived improvement in diagnostic capability over singular parameter measurement of elapsed time to clot datum produced by activated partial thromboplastin time and/or prothrombin time/INR. Previous studies have used TEG to improve ATC diagnosis or to advance knowledge of ATC mechanisms[9–11] through description of normal donor variables of RBCs, platelets, and plasma.[12] Other studies focused solely on pH-induced coagulation alteration.[13,14] However, no attempts have combined both study of acidemia and blood components to fully evaluate the possible contributions to ATC, particularly as measured by TEG.

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For the TEG, using blood from a trauma patient, to be informative and to guide subsequent therapy for ATC, one must consider some hematologic events occurring during traumatic hemorrhage. First, there are significant decreases in circulating RBCs because blood loss and compensatory shifts of interstitial fluid into the vascular compartment.[15] Such decreases are reflected by hematocrits (Hcts) as low as 30%.[16] Second, because of tissue hypoperfusion and resulting hypoxia, there is increased blood lactic acid, which results in acidosis.[17] Reduction in pH decreases coagulation activity at numerous levels, one of which is evidenced in vitro by a 90% decrease in factor VIIa activity and a 55% decrease in factor VIIa/TF complex activity at pH 7.0 compared with physiologic values of pH 7.4.[18]

In addition to the previously noted hematologic events, one should also consider that blood used for Hct and TEG measures is usually collected from a large vein or artery. Such TEG data therefore represent conditions in large vessels of the systemic circulation (1% of the circulatory system). However, bleeding phenomena associated with ATC occur in the microvasculature where Hct values are as low as 10%,[19] and acid production occurs as a result of hypoperfusion and hypoxia.

With all such considerations in mind, the objective of the current studies was to characterize TEG results under conditions that more closely reflect those occurring in microvascular blood during traumatic hemorrhage. We hypothesize that reduced pH in combination with low Hct values found in that environment will result in TEG data that are significantly different from TEGs of blood collected from larger vessels, where Hcts are higher. We suggest that the TEG values obtained by modeling acidemia in lower Hct blood are more indicative of true decreases in coagulation function. We further hypothesize that dilution of blood-induced coagulopathy, via theoretical Starling fluid-flux mechanisms or crystalloid infusion, occurs at the microvascular level yet cannot be diagnosed through current TEG methods of WB analysis. Such models may ultimately guide improvements in resuscitation of ATC.

PATIENTS AND METHODS

Donor Selection

Healthy males were recruited for blood donation and advised according to a protocol approved by the US Army Medical Research and Materiel Command Institutional Review Board and in accordance with the Helsinki Declaration on patient safety. Upon consent, donors agreed to have taken no medications that alter coagulation function. Blood samples were obtained from a donor pool that allowed even distribution across all treatments, and hence, any measured effects cannot be due to a unique donor.

TEG Assay—Component Separation

For each experiment of blood component separation or simulation of resuscitation with plasma or normal saline (NS), fresh phlebotomy WB was drawn from one donor of a selection pool into citrate vacutainers (final, 10.9 mM) and further contact coagulation pathway inhibited with 100-μg/mL corn trypsin inhibitor (Haematologic Technologies, Essex Junction, VT). A 10-minute 200-g centrifugation at 25°C yielded platelet-rich plasma (PRP). A subsequent 20-minute 2,000G centrifugation at 25°C of the remaining blood yielded platelet-poor plasma (PPP) and erythrocytes (RBCs). PPP and RBCs were stored briefly on ice. Donor WB was compared with 40% Hct RBC/PPP as well as groups containing no RBCs: PRP and PPP.

TEG Assay—Variable Hct

RBC effects on coagulation were investigated in the presence of an acidic buffer volume titration (explained in the following section) using variable Hct (40%, 30%, 20%, and 0%) while holding platelet concentration at physiologic values (donor specific). Control of Hct was accomplished through replacement of RBC volume with an equal volume of plasma that simulated the decreasing Hct transition from large vessels to small. Differences in simulated resuscitation were investigated using similar RBC replacement methods with NS that maintained plasma levels at 60% yet produced controlled and variable Hct.

TEG Assay—Experimental Design

Our design used a predetermined spectrum of pH 7.4, 7.1, 6.8, and 6.7 in WB. pH 6.7 has been experimentally witnessed in acidotic tissue of animal hemorrhagic shock models within 30 minutes in a 45-mm Hg pressure hemorrhage model.[20] Therefore, although pathologic systemic pH rarely drops to less than pH 7.1, our simulation spectrum was bracketed on the low end by pH 6.8 for local hypoperfused tissue pH consideration. These pH values were generated by adding increasing volumes (0 μL, 3 μL, 6 μL, and 10 μL) of 1-M 2-[morpholino]ethanesulfonic acid–buffered saline (MBS) to a 300-μL volume sample in the TEG cup (Table 1). MBS was chosen for its previous use in similar experiments.[18] Although a spectrum of measured volumes were added to blood samples, all represent less than 5% dilution of the total sample volume.

PRP and PPP achieved lower pH values than did WB because of RBC buffering effects. In all citrated blood TEG experiments, 37°C blood components were premixed with 1:5,000 Innovin (recombinant 1–243 TF with phospholipids

TABLE 1. MBS Titration to Establish pH 7.4, 7.1, 6.8, and 6.7 Within WB Samples

	WB		PPP	
1-M MBS, μL	pH	Bicarbonate	pH	Bicarbonate
0	7.27 ± 0.02	22.53 ± 0.72	7.35 ± 0.04*	21.68 ± 1.01
3	7.06 ± 0.02	18.18 ± 0.49	6.85 ± 0.02*	17.85 ± 0.84
6	6.84 ± 0.01	13.53 ± 0.54	6.59 ± 0.01*	9.80 ± 0.80*
9	6.67 ± 0.01	9.63 ± 0.31	6.43 ± 0.01*	No data
12	6.53 ± 0.02	No data	6.31 ± 0.01*	No data

*Significant differences between WB and PPP values (pH or bicarbonate) ($p < 0.05$).

Titration of 1-M MBS stock into citrated WB or centrifuged and isolated citrated PPP resulted in declining pH and bicarbonate values (mean ± SEM; n = 4). The microliter values of 1-M MBS listed in the first column are representative of the injection volume necessary to achieve the WB or plasma pH listed in experiments using TEG cups that contain experimentally designated 300-μL of blood product. Bicarbonate values that were below the limit of detectability are reported as "no data."

[TF/PL], Dade-Behring, Marburg, Germany) and immediately added in 300-μL aliquots to TEG cups containing 4.5-μL 1-M $CaCl_2$ (final, 15 mM) and appropriate volume of MBS acidification buffer solution.

Statistical Analyses

Statistical analyses of TEG data made use of a combination of Microsoft Excel, JMP version 10.0, and GraphPad Prism 5.0 software. Routinely, a two-way analysis of variance of data was conducted followed either by Tukey's HSD test (data involving different blood products and volume of MBS added; n = 4) or with Fisher's least significant difference test (data for variable Hct and volume of MBS added; n = 5). TEG parameters chosen for analysis were as follows: (1) "split point" (SP), the first signal noted by TEG software and due to nascent thrombus formation; (2) reaction time (*R* time), defined as the time from the start of the sample run to achievement of 2-mm amplitude in the TEG tracing; (3) angle, reflects the kinetics of clot development; and (4) shear elastic modulus strength (SEMS), expressed in dynes per square centimeter, a representative measure of clot strength. All data are presented as mean ± SEM.

RESULTS

TEG Responses to Increasing Volumes of MBS With and Without Presence of RBC

With increasing volumes of MBS (MBSvol) added to PPP and PRP, a prolongation of SP (Fig. 1*A*) and *R* time (Fig. 1*B*) occurred. No significant increases in SP or *R* time were measured within RBC/PPP or WB groups with greater MBSvol (Fig. 1*A* and *B*). Effects of MBSvol on angle measurements (Fig. 1*C*) were significantly different only in the PPP group. Inclusion of RBCs (note WB and RBC/PPP groups) eliminated the effects of acidemia on all TEG measures (Fig. 1*A–D*). SEMS differences (Fig. 1*D*) are observed only when comparing varying MBSvol in the PRP group. (all groups; $p < 0.05$ with the exception of native PRP vs. the 3-μL MBSvol [$p = 0.136$]).

TEG Measures: Effects of RBC and Dilution Medium

Based on the previously presented TEG responses with different amounts of RBC, further experiments using either PPP or NS replacement of RBC were conducted. PPP replacement of RBC begins to cause significant increases in SP (Fig. 2*A*) and *R* time (Fig. 2*C*) at the level of 20% Hct (0-μL MBS vs. 10-μL MBS). At 0% Hct, increases of greater significance are realized between most of all comparisons of MBSvol. With NS replacement of RBC, effects similar to those in Figure 2*A* develop. SP (Fig. 2*B*) and *R* time (Fig. 2*D*) are prolonged following increasing MBSvol and decreasing Hct. However, greater increases in SP (Fig. 2*B* vs. 2*A*, 10-μL and 6-μL MBS, $p < 0.0001$) and *R* time (Fig. 2*C* vs. 2*D*, 10-μL and 6-μL MBS; $p < 0.0001$ and $p = 0.0005$, respectively) occur with NS versus plasma replacement of RBCs when increasing MBSvol in the 0% Hct group only. At 0% Hct, increases are significant among all MBSvol comparisons with the exception of 0-μL MBS versus 3-μL MBSvol ($p = 0.329$). At 0% Hct in the saline replacement group (Fig. 2*D*), the divide between *R* time of the 0-μL

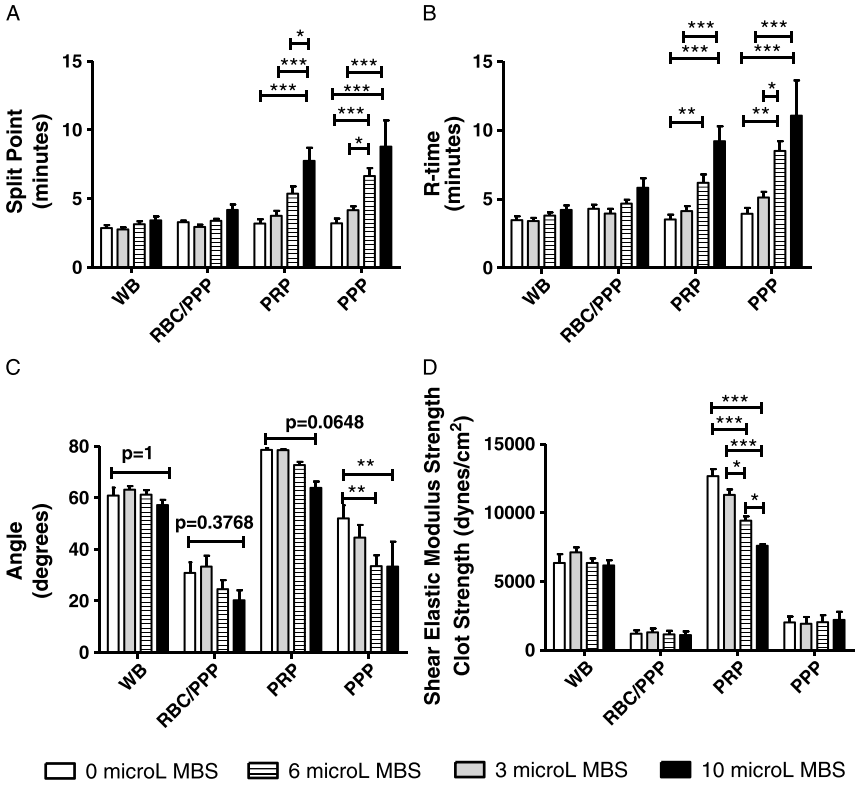

Figure 1. TEG evaluation of equal TF/PL activation of various blood components and combinations (WB, RBC/PPP, PRP, and PPP) was conducted with a titration of 1-M MBS. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$; n = 4.

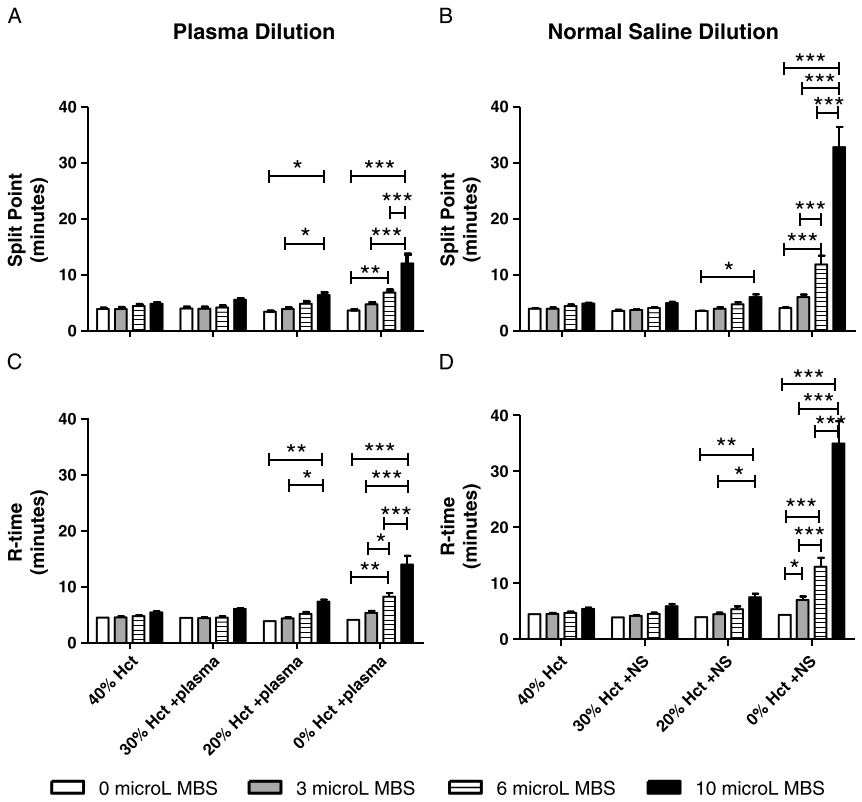

Figure 2. TEG SP and *R* time evaluation of equal TF/PL activation in an acidemia model of decreasing Hct with either plasma replacement (*A* and *C*) or NS (*B* and *D*) of RBCs. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$; n = 5.

MBSvol versus the 3 μL MBSvol group becomes significant. Irrespective of replacement/dilution liquid, increasing MBSvol had no effect on SP or *R* time when RBC were in sufficient amounts to provide a Hct of 30% or greater (Fig. 2*A–D*). As dilution continued from Hct of 20% and 0%, increasing MBSvol prolonged both SP and *R* time ($p < 0.05$), and this effect was more pronounced when dilution occurred with NS (Fig. 2*B* and *D*) instead of plasma (Fig. 2*A* and *C*).

Irrespective of HCT or dilution liquid, increasing MBSvol decreased ($p < 0.05$) TEG angle within each percent Hct group (Fig. 3*A* and *B*). However, at 0% Hct, differences in TEG angle were most dramatic with 35% and 44% decreases occurring with plasma or saline dilution, respectively (Fig. 3*A* and *B*) between normal and the highest MBSvol group. SEMS data were complex (Fig. 4*A* and *B*). Increasing MBSvol had no effect on SEMS at 30% Hct with plasma diluent but decreased SEMS at that Hct with a saline diluent. At 20% Hct, increasing MBSvol decreased SEMS in the presence of either diluent (Fig. 4*A* and *B*). When plasma was used as a diluent to achieve 0% Hct and in the absence of added MBS, SEMS were 2.23-fold greater than at 40% Hct and was significantly greater than all other Hct values (Fig. 4*A*[a], $p < 0.0001$). This increase, although equally significant, was much less with saline diluent (Fig. 4*B*[a], 1.60-fold; $p < 0.0001$). Indeed, SEMS at 0% Hct and with no added MBS in the saline diluent were 28% less ($p < 0.0001$) than those with plasma diluent. For both diluents, increasing MBSvol was associated with decreased SEMS (Fig. 4*A* and *B*).

DISCUSSION

We hypothesized that TEG measures of coagulation using blood taken from large blood vessels of the systemic circulation do not accurately reflect coagulation processes, which occur in microvessels. Moreover, ATC results from trauma-induced changes in coagulation in these microvessels. Hence, the logical corollary that permeates our hypothesis is that the previously noted TEG measures do not provide accurate information with which to understand and ultimately treat ATC. The purpose of these studies was to address this hypothesis and its corollary using an in vitro system to mimic microvessel coagulation physiology. Four main findings from this study support our hypothesis. (1) Individual blood components reflect altered coagulation activity when analyzed via TEG under increasingly acidic conditions. (2) For all measures (SP, *R* time, angle, and SEMS), RBCs supply resilience to acidic alteration of coagulation (Figs. 1 and 2*A* and *B*). (3) Significant loss of coagulation activity occurs as a result of an acidic environment at various Hct concentrations used to mimic different levels of the vascular tree (Figs. 2–4). (4) Our simulated coagulation parameter perturbations of acidemic microvascular blood following plasma resuscitation are exacerbated in the scenario of NS resuscitation solution substitution; these anomalies are masked through the use of current TEG evaluation methods.

RBC buffering effects on blood plasma pH are effected through RBC associated carbonic anhydrase, [H^+] binding to

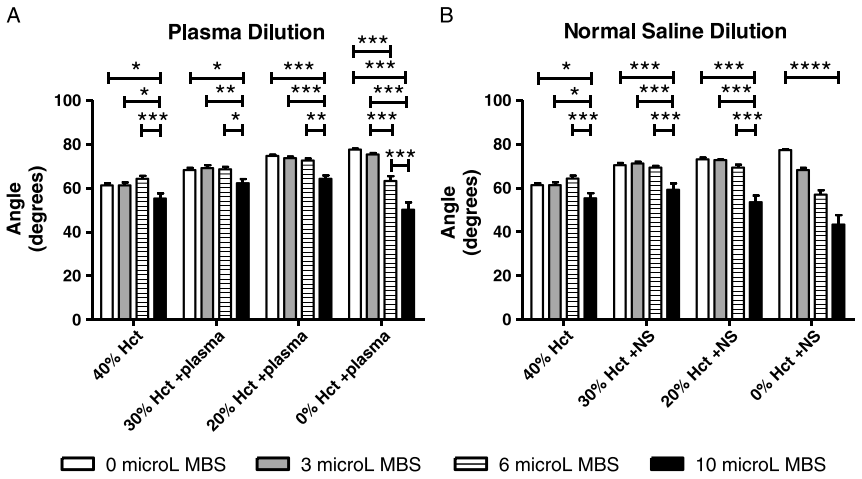

Figure 3. TEG angle evaluation of equal TF/PL activation in an acidemia model of decreasing Hct with replacement of RBCs with either plasma (*A*) or NS (*B*). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ***$p < 0.001$ throughout all acid load comparisons (0% Hct [*B*]); n = 5.

hemoglobin, potassium exchange, and bicarbonate release.[21] However, the purpose of this article was not to further investigate RBC buffering effects. Metabolic acidosis is not immediate following trauma. Yet, an almost immediate production of lactic acid occurs in ischemic or hypoperfused tissues that cannot be measured in systemic circulation blood samples. Table 1 shows obvious significant differences in pH when comparing WB versus PPP presumably caused primarily by the influence of RBC. Bicarbonate values trended down in the PPP group faster than in the WB group yet only showed significantly lower values at the 6-uL added MBSvol pairwise comparison.

The underlying foundation of this study is that the site of acid generation is in the damaged tissue. Therefore, we attempted to simulate coagulation function at this site by altering the concentration of RBC (Hct) in the presence of increasing [H^+]. The literature reports pH as low as 6.2 in ischemic tissue,[22] whereas pH less than 7.2 is rarely seen in systemic blood samples. The relative contribution of local versus systemic influence on the ATC phenomenon is unknown but may change with time according to the severity of the wound.

Current evidence suggests that TEG and rotational thromboelastometry are valuable tools in managing trauma patient coagulopathy.[23,24] Insufficient clot strength and hyperfibrinolysis have been identified as factors that independently affect mortality.[25,26] However, the literature reports INR ratios to be more sensitive early-detection measures of increased odds for mortality.[6,27] INR values closely correlate with TEG parameters SP and *R* time, yet the absolute distinction between the two methods of coagulation assessment (INR vs. TEG) is the use of WB (in TEG testing) or the isolated plasma component (in INR testing).

The presence or absence of RBCs must be considered when performing diagnostic tests of coagulation. RBC effects are complex such that an RBC deficit that directly promotes a bleeding diathesis may be attributable to more than simple loss of promargination effects upon flowing platelets. Historic studies show anemic patients to have a prolonged bleeding time (BT) that was correctable with RBC infusion.[28] In another study, acute loss of RBC contributed to nonsurgical bleeding as shown by a strong correlation between low admission Hct,

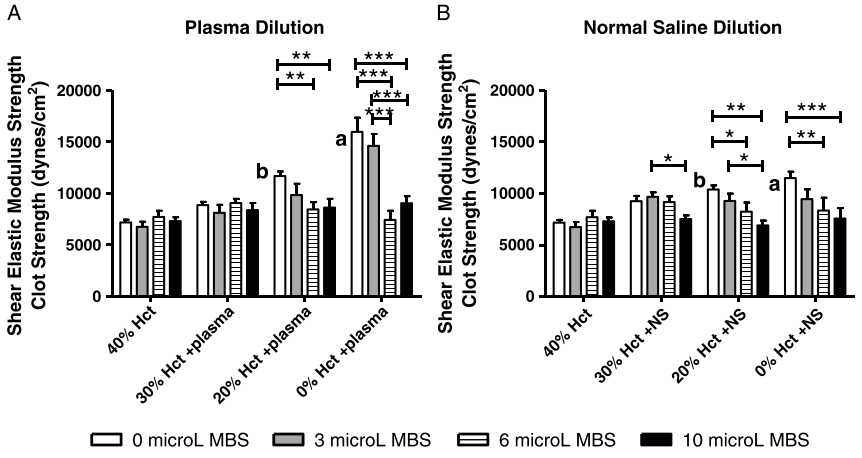

Figure 4. TEG clot strength evaluation of equal TF/PL activation in an acidemia model of decreasing Hct with replacement of RBCs with either plasma (*A*) or NS (*B*). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$; n = 5.

packed RBC transfusion, and 24-hour blood product requirements.[16] However, in a primate model that investigated thrombus formation in a collagen containing shunt with variations in Hct between 20% and 55%, no correlation was found between clot volume and Hct at high-flow condition (750 per second), and a negative correlation was found in low-flow condition (100 per second).[29] BTs were within normal range for all groups, yet for the low-Hct group, BT was significantly prolonged compared with the normal- and high-Hct groups. As suggested by the primate model example, data collected from study of coagulation in larger vessels of greater than 20% Hct may not be relevant to pathologic hemostatic failure in microvessels as is examined via BT testing.

Wohlauer et al.[30] findings suggest that hemodilution is not critical in the absence of acidemia. Although our dilution methods differed (rat model with 50% NS hemodilution: approximately 20% Hct), it is easy to see that dilution with plasma shows much greater clot strength than does NS dilution with no pH alteration when considering low Hct values representative of microvascular beds (Fig. 4A^a vs. B^a, $p < 0.001$). It is however interesting that our findings are in agreement with the study of Wohlauer et al. that at 20% Hct, no significant differences exist between plasma and NS dilution (Fig. 4A^b vs. B^b, $p = 0.23$). Experimentally increased acid load further prolongs clot times and decreases clot strength in our TEG analysis of simulated microvascular blood (20% and 0% Hct).

It is of interest to note the dramatic increase in clot strength that occurred with plasma diluent and in the complete absence of RBC. This undoubtedly reflects an increase in concentration of plasma coagulation factors (including fibrinogen) that allows for a much greater SEMS. It is also of interest to note that only under conditions of reduced RBC (20% and 0% Hct) did increased acidity lower measurable clot strength. Such data in this in vitro model, again, suggest the possibility that TEG testing of blood from a major vessel does not accurately reflect conditions in microvessels. Such inaccuracies contribute to our inability to adequately characterize ACT via use of TEG.

There are potential limitations in this study. This study design distinguishes the systemic blood studied for ATC diagnosis and the actual extreme coagulation disturbance that would exist in microvascular bed bleeding. However, when considering this same model for centrifugation of trauma patient blood for component analysis, PPP and PRP pH will likely have been corrected through temporary association with RBCs, which has not been described by this study. Criticism may come with the fact that no TEG studies truly simulate the capillary environment. We agree our findings are of correlative value as capillaries supply an overwhelming anticoagulant surface that may completely prevent coagulation if the endothelial structure remains intact.[31]

TEG is attractive for clinical use because it lends itself to study of all blood components. In this study, our optimized TEG procedure offers differentiation capabilities between blood elements and associated coagulation parameters. However, TEG values reflect strength and stability of the clot as produced in a closed reaction system with TF/PL uniformly distributed throughout the sample, a scenario reflective of thrombosis rather than hemostasis. TEG is not a measure of thrombin generation. In fibrinogen-depleted samples, no signal is produced. In patients with consumptive coagulopathy, TEG tracings are significantly reduced in amplitude due primarily to lesser fibrinogen levels and platelet counts. These physical parameters may help characterize clots produced but only allude to hemostasis, which is the maintenance of vascular integrity in an open system of continuous supply of platelets, zymogens, and inhibitors. In patients with various factor concentration or activity deficits such as hemophilia A and B, the vast differences in TEG parameters observed in comparison with a normal population translate well from deficiencies in thrombosis to deficiencies in hemostasis. Deficits or excesses observed in ATC may be of the same spectrum of alteration but are multifaceted and unlikely to be fully interrogated through TEG analyses. Civilian trauma patients and injured soldiers arrive to surgical care without[7] (median time injury to blood draw, 32 minutes) or with significant fibrinogen consumption[32] (90 minutes). Further division of plasma effects in quick successive time point analyses of delay of thrombin burst, magnitude of thrombin burst, and fibrinogen depletion is necessary to prove the existence of as well as define, diagnose, and appropriately treat coagulopathy of trauma and ATC. The simple improvements and parsing mechanisms described in this study can have a major influence on patient management and outcome not only for trauma scenarios but also for sepsis, burn, cardiac surgery, postpartum hemorrhage, disseminated intravascular coagulation, and pharmaceutically induced altered coagulation assessment.

Despite a large body of literature, there is little consensus on cause and treatment of ATC, coagulopathy of trauma, and massive transfusion–associated uncontrolled bleeding. Large-scale clinical studies of trauma patients[26,33–36] fail to appropriately identify or separate these pathophysiologic mechanisms partially because of a lack of a reductionist approach. Consideration of these findings from the in vivo perspective may help describe the trauma patient phenomenon of continuous oozing mucus membrane and multiabrasion bleeding. Further consideration of these findings suggests that the coagulation defects so often being corrected in the clinical setting are those occurring within the lumen of larger vessels. Such potentially inappropriate corrections could thereby possibly drive disseminated intravascular coagulation while doing little for smaller vascular bed nonsurgical bleeding of damaged tissue and organs.

AUTHORSHIP

J.E.C. designed and performed the research, analyzed the data, conducted the independent statistical analyses, and contributed to the writing of the article. J.K.A. conducted the independent statistical analyses and contributed to critical review of figures and final statistics. A.P.C designed the research and contributed to the writing of the article.

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